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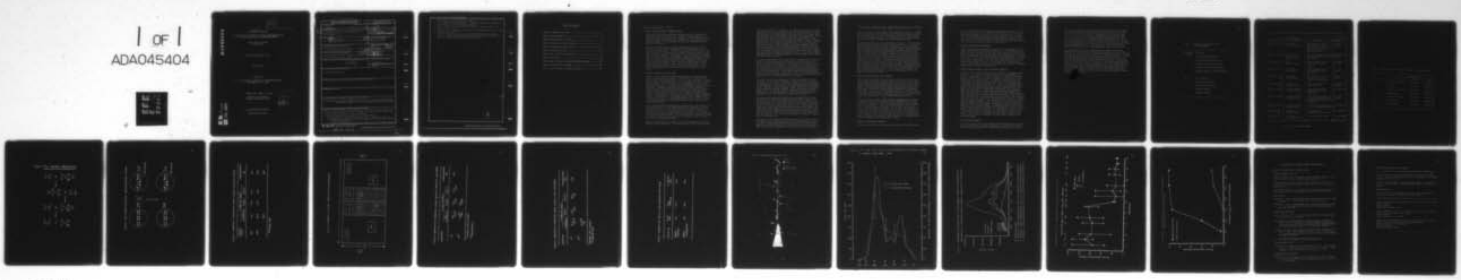

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GENETICS OF THE ENCEPHALITIS VECTOR, CULEX TARSALIS FOR POSSIBLE APPLICATION IN INTEGRATED CONTROL

Annual Report, 1976-77
(Third Year)

Sister Monica Asman, Ph.D.

May 30, 1977

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Culex tarsalis: reciprocal translocations; vector competence; computer models competition studies ; mass rearing; mutants; population dynamics

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The projects here reported represent part of an overall program designed to change Culex tarsalis genetically to inhibit its propagation in nature, and to render it less effective as a vector of disease.

A resume of progress for the year 1976-77 is as follows:

A. The number of maintained strains for genetic studies was increased.

B. Multiple-marker strains for genetic studies and identification of translocations increased to 7.

C. Among the translocation strains that show promise are: 9 sex-linked,

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3 autosomal. Two sex-linked are multiples, involving all 3 chromosomes. Two autosomes are in homozygous condition.

D. A sex-linked multiple translocation strain was studied for competitiveness in laboratory and large-outdoor cages.

E. Computer models were used to determine number of males released this spring in pilot study.

F. A mass-production program successfully produced 100,000 males over a predetermined time period.

G. In vector competence studies, a refractory strain was successfully selected. Genetic studies determined that susceptibility to WEE virus was dominant and controlled by more than 1 gene.

TABLE OF CONTENTS

Annual Progress Report 1976-77

A. Culex tarsalis strains and mutant lines

The number of wild-type laboratory colonies currently maintained in our laboratories is 11 (Table 1). Another strain, Berkeley, is a composite strain which holds some genotype of several California lines. The single mutation strains total 11 (Table 2), and strains holding multiple-markers -- one marker on each of the 3 chromosome pairs -- now total 7 (Table 3).

The mutant charcoal (char), already described last year in genetic crosses, was shown to be sex-linked, as is wide wing (ww), a more recently isolated mutant. The latter was selected out from the fringe (fr) mutant line. Wide wing gives a broader than normal width to the wing, primarily because of an extended lobe on the posterior wing margin. It also shortens the wing-length. Another new mutant is clubbed palp (cp) which is expressed by abnormal palps in the males only. To date its linkage relationship has not been determined. Orange body (orb) was recently isolated from a Presidio, Texas strain; however, its linkage relationship has also not yet been determined. It is expressed by giving a pale orange color to the fat bodies of larvae, pupae, and adults, and is more expressive in the females than the males.

B. Induced reciprocal translocations

While our total induced chromosome interchanges have numbered over 27, we are presently maintaining 12 translocation strains that show potential for control mechanisms. Of these 9 are sex-linked -- 6 single and 3 multiple -- interchanges, and 3 are autosomals involving linkage groups 2 and 3. Two of the autosomal translocations are now in the homozygous condition. In several of the sex-linked translocation lines, two types of abnormal segregation were found to occur irregularly. Each type is a meiotic drive system resulting in a surplus of recombinant progeny. In addition, a sex switch mechanism was found to operate in several lines (Figure 1). In essence an M-linked translocation is transmitted from a male (m/M) to a daughter (m/m), apparently through a recombination. The daughter transmits the translocation to a portion of her sons, as an m-linked translocation. These sons then transmit the translocation almost exclusively to their sons, as an M-linked translocation.

One of the sex-linked translocations, designated T(1:2:3)a carries two interchanges that in pseudo-linkage binds the 3 chromosomes involved and transmits these as a set from males to male progeny (Figure 2). The line is easily maintained by using genetic markers, and appears to be strong and viable. The mean percent egg hatch of rafts fathered by these males is between 20-30%. For these reasons this strain was tested for competitiveness against wild males in both small-cage laboratory and large-cage outdoor experiments.

The first competitive mating tests were done in laboratory cages under optimum rearing conditions. The cages were 24"x24"x20", and each cage

held 50 ♀♀ and 100 ♂♂. The control cage held only the Knights Landing laboratory strain (or the black-eye line in the second trial), another cage held only the translocation stock, and a third competition cage held 50 normal ♂♂ with 50 translocated ♂♂ and the 50 normal ♀♀. Twenty-five egg rafts were collected from each cage, and competitiveness between the 2 types of males was judged from egg-hatch data. This is possible since normal egg rafts usually have above a 75% hatch and the translocation line usually has under 30% hatch. The data (Figure 3) show that the hatch in the competition cages was in-between the two expected egg hatches, and the competitiveness (E) derived from known factors in formula, gave 2 values greater than 1. These data suggest that experimental males were a little more competitive than the normal. One value, below 1, showed the opposite effect. In any case the data determined that the genetically-altered ♂♂ could compete nearly equally with the laboratory strains.

To test the experimental stock under natural environmental conditions, we used a modified quonset hut located near our Bakersfield Field Station in Kern County (Figure 4). This structure is divided in half, and is covered with a fine-mesh screen except for a solid section in the center that provides shade. Each section has artificial ponds for oviposition, cages that hold chickens to provide blood meals, and a "red box" resting site. A small cage in the competitive side held only our translocated line. Each half of the "hut" held 1000 normal ♀♀; the control side held 2000 normal ♂♂, and the competition side held 2000 ♂♂ in a 1:1 ratio of normal to translocated ♂♂.

The first test involved translocated ♂♂ competing against a strong laboratory colony, Knight's Landing. The data (Figure 5) indicated a competitive value of 0.73, indicating that, under these conditions, the experimental ♂♂ were a little less competitive than the laboratory strain. However, the percent-hatch of eggs collected in the 2nd generation was lower than was expected. Thus, not only were the experimental ♂♂ able to compete, but the translocation was successfully transmitted to the next generation. This was confirmed by further laboratory tests on ♂♂ that came from the low-hatch rafts.

The second trial tested the translocation ♂♂ against ♂♂ collected as pupae from the West Poso Creek site in Kern County where we are doing our first pilot field release study this year. Again, as the data show (Figure 6), the percent-hatch was about midway between the normal, 96.0 and the translocated control, 50.8. Both percent-hatch figures were higher than expected, and the reason is not known. The competitive value, however, was again 0.76, and the reduced percent-hatch that carried through to the second generation was again lower than the expected. Both values indicated that a single insertion of the translocated ♂♂ at a 1:1 ratio could affect not only the first but also the 2nd generation.

A 3rd trial in the modified quonset hut merely held control tests on both sides of the structure, to ascertain if any differences in data were due to positioning the North or South side of the large cage. Earlier data showed variability in oviposition and adult mortality. The percent-hatch was approximately the same on both sides (Figure 7),

as was the total number of eggs, although the variability was obvious for the number of rafts and mean number of eggs per raft on the 2 sides.

Since we had only 1 large divided cage available in 1976, the 3 trials were necessarily done under different environmental conditions because of the time of year. The first tests were run in June and July and the last in September-October. A second large cage is now available so that more trials are being done simultaneously this year.

As mentioned above, two of the translocation strains are now homozygous and with time are becoming stronger and more prolific. A selection process in one line (T(2:3)a) from a high-yield viable raft appears to be improving this pure-breeding autosomal stock. This translocation and the multiple sex-linked translocation described above have now been combined in males. Computer simulations show that the introduction of such multiple translocation males has a potential of being more effective in field populations than does the double sex-linked interchange complex alone. While the sex-linked translocations will be passed on to the males of subsequent generations, the autosomal interchange will be passed on to females in the population and thus add to the reduction of numbers in viable progeny. This new multiple translocation male, carrying the double sex-linked translocations and the autosomal interchange, will be tested in our large outdoor cages for competitiveness this summer. If the results are favorable we will introduce this interchange into a field population as a pilot study in the summer of 1978.

C. Marked-release-recapture field studies

While competitive studies were on-going in the large outdoor cages, an extensive mark-release-recapture study was carried out from April through September at the West Poso Creek site by collaborating personnel at the Bakersfield Field Station. The site is near the field station and the modified quonset "hut" where the competition tests took place. The site itself is isolated by extensive surrounding arid desert (Figure 8), and the mosquito population is almost solely C. tarsalis. The creek water supply is stable as it is supplied by waste waters from oil wells. Preliminary data were collected in 1975 on the rise and decline of this isolated natural population.

During the summer of 1976, 20,845 marked C. tarsalis were released to provide a basis for evaluation of population numbers and adult longevity in the field. In 48 nights of intensive recovery effort, 4.4% were recaptured. A total of 134,965 mosquitoes were collected from the 24 traps and 17 red boxes. Data of this kind not only helped determine population densities at specific time periods, but supplied the necessary information for projection of the numbers of experimental ♂♂ that should be released in the pilot-release study in the spring of 1977. The data have been placed in a computer program for fitting to theoretical models (Figure 9). The mark-release studies are being repeated this summer (1977).

D. Computer modelling for releases

In the past year Dr. Paul Fine developed computer models with refer-

ence to alternative specific genetic control systems that may be used for population control. The simulation which will be followed in our first release trial is based on the release of males that carry the T(1:2:3)a sex-linked double translocation, and this is seen in Figure 10. This model illustrates the result of releasing 10,000 ♂♂ every 3 days over a one month period, and assumes that the experimental males are competitive and equally viable to wild-type ♂♂.

E. Polytene chromosome preparations

Techniques for preparing polytene chromosomes in C. tarsalis are still being investigated, and progress has been made. The salivary-gland polytenes are being spread to the extent that pictures of sections and whole chromosomes can be photographed so that centromeres can be recognized and individual patterns of banding can be established for specific chromosomes. Once normal chromosomes are recognized, we will be able to ascertain cytologically where the chromosomal breaks occur in the translocations induced, and if other anomalies are contributing to zygotic inviability. Good preparations would also allow us to establish linkage-group chromosomal correlations.

F. Vector-competence studies and the genetics involved

The studies involving the selection of a strain of C. tarsalis highly resistant to oral infection with Western Equine Encephalitis virus during 17 generations can be seen in Figure 11. Figure 12 shows the profiles obtained with the highly susceptible and refractory strains when feeding on viremic chicks. A completely refractory strain of C. tarsalis could not be selected since 15-20% of the females that fed on high concentrations of virus were found to be infected after an intrinsic incubation of 10-12 days. Also it was found that the resistant and susceptible strains were equally susceptible when the midgut was by-passed by intrathoracic inoculation. This suggested that some females might become infected by means other than those under genetic control. Genetic backcrossing studies between susceptible and refractory lines supported this contention. The "leaky gut" concept derived from studies of other viruses in insects was considered to be involved here, since it is conceivable that ingested virus might enter the hemocoel through a leaky midgut without first having multiplied in midgut epithelial cells. If this occurred, then infection would be similar to intro-thoracic inoculation. Further studies will hopefully clarify these findings. In genetic studies the exact mode of inheritance was not established for susceptibility or refractoriness; however, susceptibility was found to be dominant over resistance and to be controlled by more than one gene. Definitive genetic studies cannot be done until we establish the significance of females that contain intermediate concentrations of virus after feeding on high viral challenges.

G. Mass rearing processes

In order to do our first pilot-release studies beginning in the spring of this year (1977), a production program was initiated in November 1976 to assure a production of 10,000 translocated males every 3 to 4

days over a period of 1 month. Since the experimental stock carrying the double sex-linked translocation has such low fertility (20-30%), and only males were to be used to expand the stock, it was no small operation to obtain a total of the approximately 100,000 males that were released into an isolated population at our release site this past April (1977). We will be monitoring the population for the rest of the summer months to see what our release did in terms of affecting the native population, and to ascertain if our released males did indeed mate with wild-type females. We did succeed in mass-producing the experimental males for this study and that in itself was a triumph.

Our main problem in the mass-producing study, other than the low fertility of the egg hatch, was the manual sexing process which was necessary to isolate the males carrying the translocations from possible recombinants among the males and from the non-carrying females. Culling with markers was necessary each generation except in the last to be sure our translocation was being carried into the field. We know now that very little recombination took place. We are now searching desperately for a female lethal to incorporate into the experimental line so that we might have only males coming through to the adult stage in each generation of the expansion program. These males, heterozygous for the lethal linked to the m locus, can then be mated to females heterozygous for the lethal each generation, causing females to become homozygous and inviable.

Table 1. Laboratory maintained colonies of *Culex tarsalis*

California strains	Riverside
	Poso Creek (Kern County)
	Bakersfield-BFS (Kern County)
	Knights Landing (Yolo County)
	Sacramento Valley (Butte County)
	Berkeley (Hybrid of several strains)
Other	Yuma (Arizona)
	BFS-Winnipeg (Canada)
	Fort Collins (Colorado)
	Presidio (Texas)
	Manitoba (Canada)

Table 2. Monofactorial mutations that have been established as laboratory colonies.

Mutant (symbol)	Mutagenic agent and colony source	Description	Linkage*
Black eye (ble)	Spontaneous Hart Park Strain	Black pigment--actually dark green under high magnification but black to naked eye--good penetrance and in both sexes	II or III recessive +
Mulberry (mul)	Ethyl methane sulfonate (EMS) Berkeley Strain	Facets of compd. eye irregular in shape--giving convex impression	sex-linked (I) recessive +
Microcephalic (mic)	EMS Berkeley Strain	Many individual facets of compd. eye completely absent--	sex-linked (I) recessive +
Carmine (car)	Spontaneous Yuma Strain	Dark red pigmented eye, seen in larvae, pupae and adults	II or III recessive +
Setaceous palps (sp)	Spontaneous Dewarts Strain	♀♀ have 1 or 2 setae on each apical segment of palps, parallel to prob.	linkage (?) recessive +
Bleached ocelli (bloc)	Spontaneous Presidio Strain	Ocelli of larvae and pupae light pink	sex-linked (I) recessive
Fringe wing (fr)	Co-60 irradiation Berkeley Strain	Wing scales heavy and ruffled giving fringe appearance	sex-linked (I) +
Charcoal (char)	Co-60 irradiation Berkeley Strain	White scales on proboscis, legs and antennal pedicel missing--also reduced white on abdomen	II or III recessive +
Wide wing (ww)	Co-60 irradiation Berkeley Strain	Wing wider than usual due to lobe on posterior margin	sex-linked (I) +
Clubbed palp (cp)	EMS treated Berkeley Strain	1 or both palps clubbed at distal segment	Not determined
Orange (orb)	Spontaneous Sacramento Strain	Fat bodies light orange	Not determined

* + or - value for linkage studies

Table 3. Multiple-marker lines now available for genetic studies.

	Chromosomes		
	I	II	III
1.	sex (gene determined)	black eye	carmine eye
2.	fringe (fr)	black eye	carmine eye
3.	bleached ocelli (bloc)	black eye	carmine eye
4.	mulberry (mul)	black eye	carmine eye
5.	microcephalon (mic)	black eye	carmine eye
6.	wide wing (ww)	black eye	carmine eye
7.	charcoal (char)	black eye	carmine eye

Figure 1. The Switch Mechanism

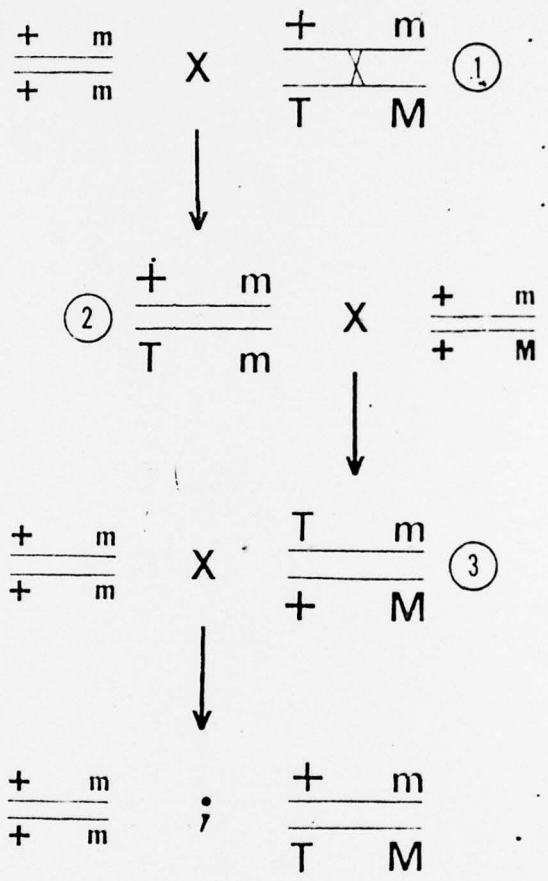


Figure 2. SEX-LINKED MULTIPLE TRANSLOCATION, T(1-2-3)a

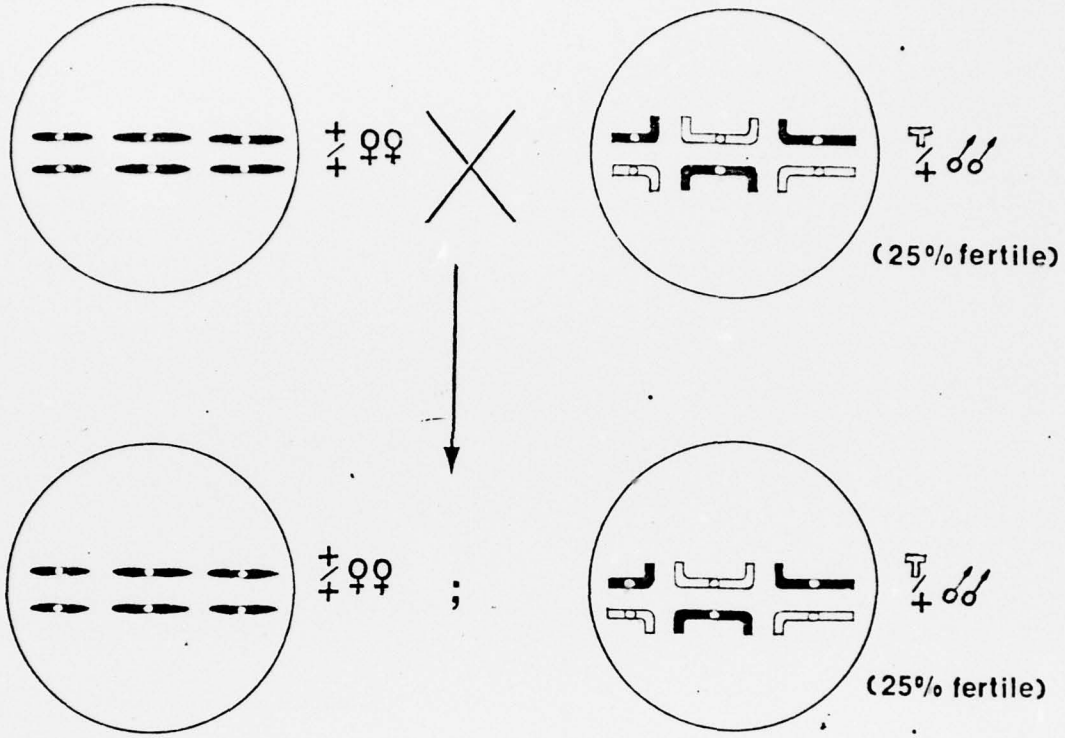


Figure 3. Laboratory competition studies against laboratory strains

mosquito strain	% egg-raft hatch*			competitiveness
	1:1 competition	normal	T(1:2:3)	(E)
ble	51.0	75.3	29.0	1.10
KLB	46.5	81.2	27.4	1.82
	60.3			0.64

*>25 egg rafts

Figure4. QUONSET HUT FOR CAGED FIELD EXPERIMENTS

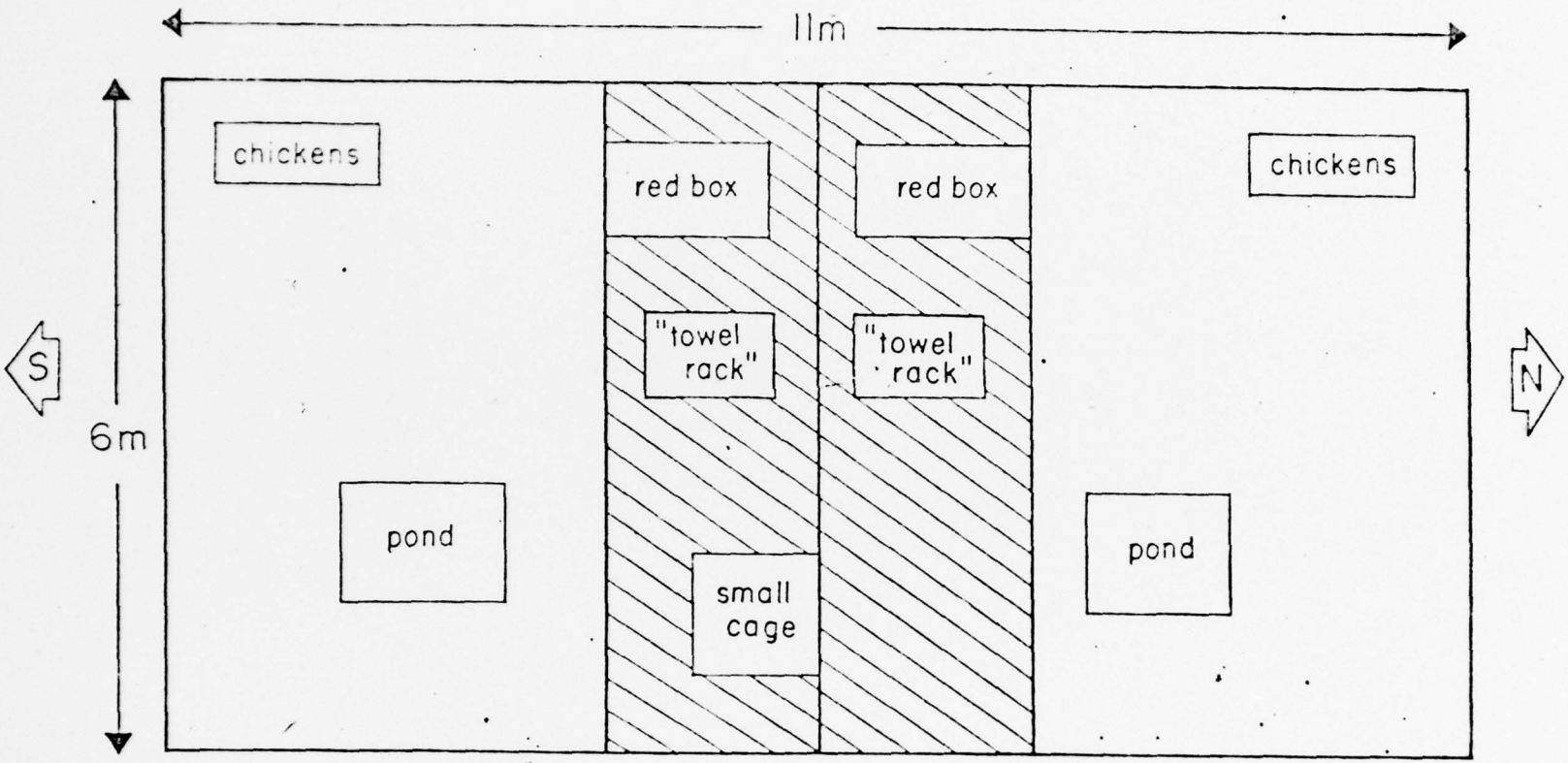


Figure 5. Quonset-hut competition studies against a laboratory strain (KLB)

generation	% egg-raft hatch			competitiveness (E)
	1:1 competition	normal	T(1:2:)a	
1st	50.1 (444)*	75.5 (122)	15.4 (25)	0.73
2nd	57.7** (100)	80.6 (100)		

* number of rafts counted

** expected = 63.5

Figure 6. Quonset-hut competition studies against a field strain(WPC)

generation	% egg-raft hatch			competitiveness (E)
	1:1 competition	normal	T(1:2:3)	
1st	76.5 (127)*	96.0 (64)	50.8 (11)	0.76
2nd	83.6** (6)			

*number rafts counted
**expected = 86.9

Figure 7. Quonset hut control using laboratory strain (KLB)

side of hut	no. of rafts	$\bar{X}$ no. eggs/raft	% egg-raft hatch
NORTH (control)	119	177.7	79.4
SOUTH (competition)	133	155.9	78.8

Figure 8. West Poso Creek relea e site

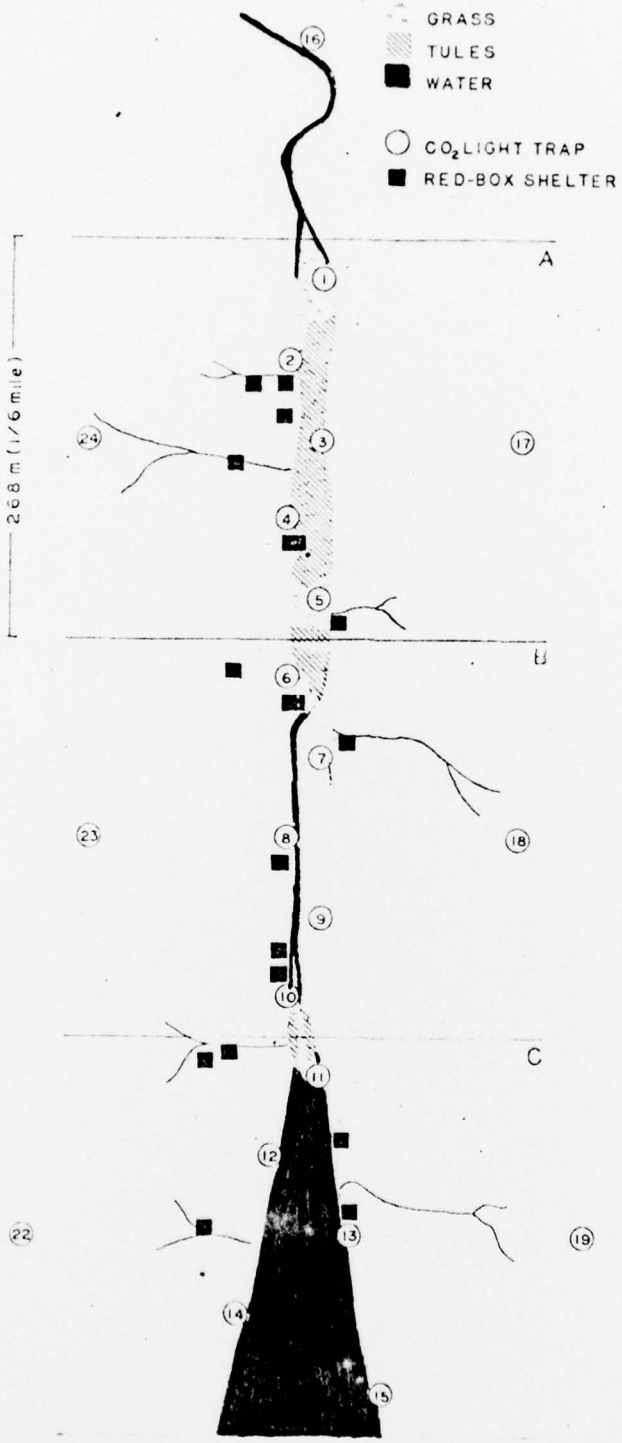


Figure 9. CDC - CO_2 light trap indices and population estimates, female *C. tarsalis*, Poso West, 1976.

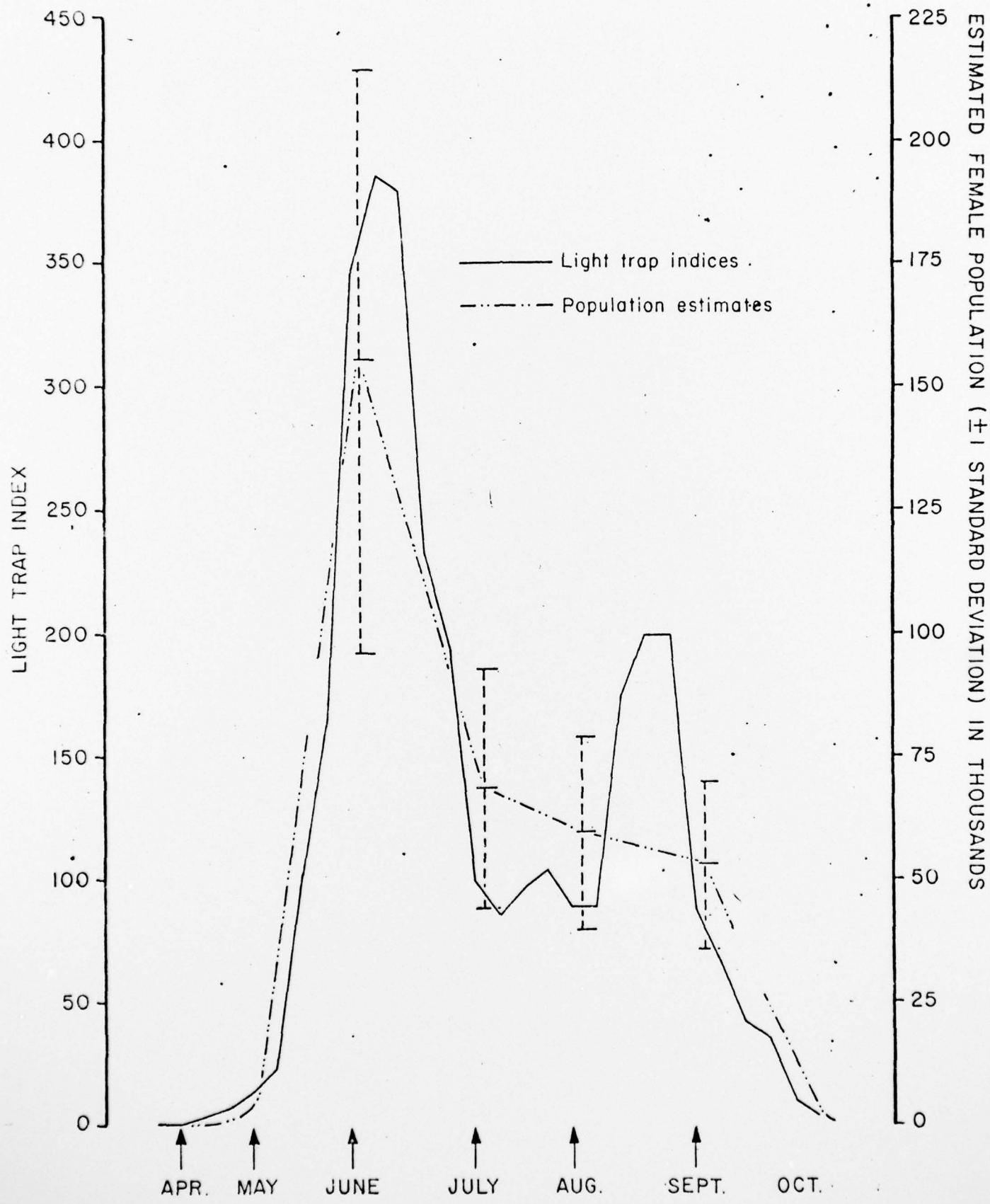
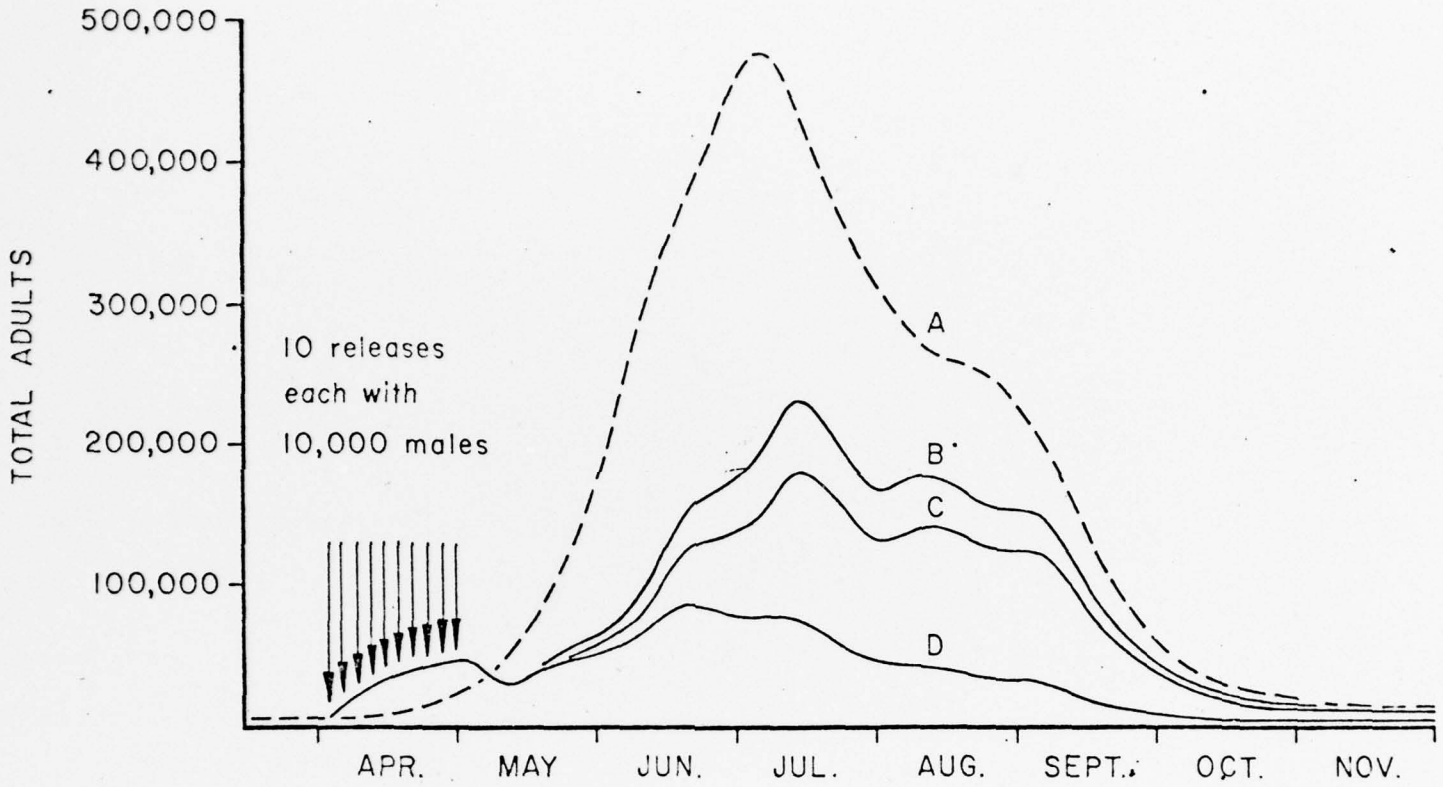


Figure 10. Computer predictions when a heterozygote double translocation is inserted into a simulated *Culex tarsalis* population at Poso West.



A. Normal population.
B. Predicted : mating competitiveness ratio 0.75, adjusted for density dependence.
C. Predicted : mating competitiveness ratio 1.0, adjusted for density dependence.
D. Predicted : mating competitiveness ratio 1.0, no density dependence adjustment.

Figure 11. Selection for refractoriness to oral infection with WEE virus

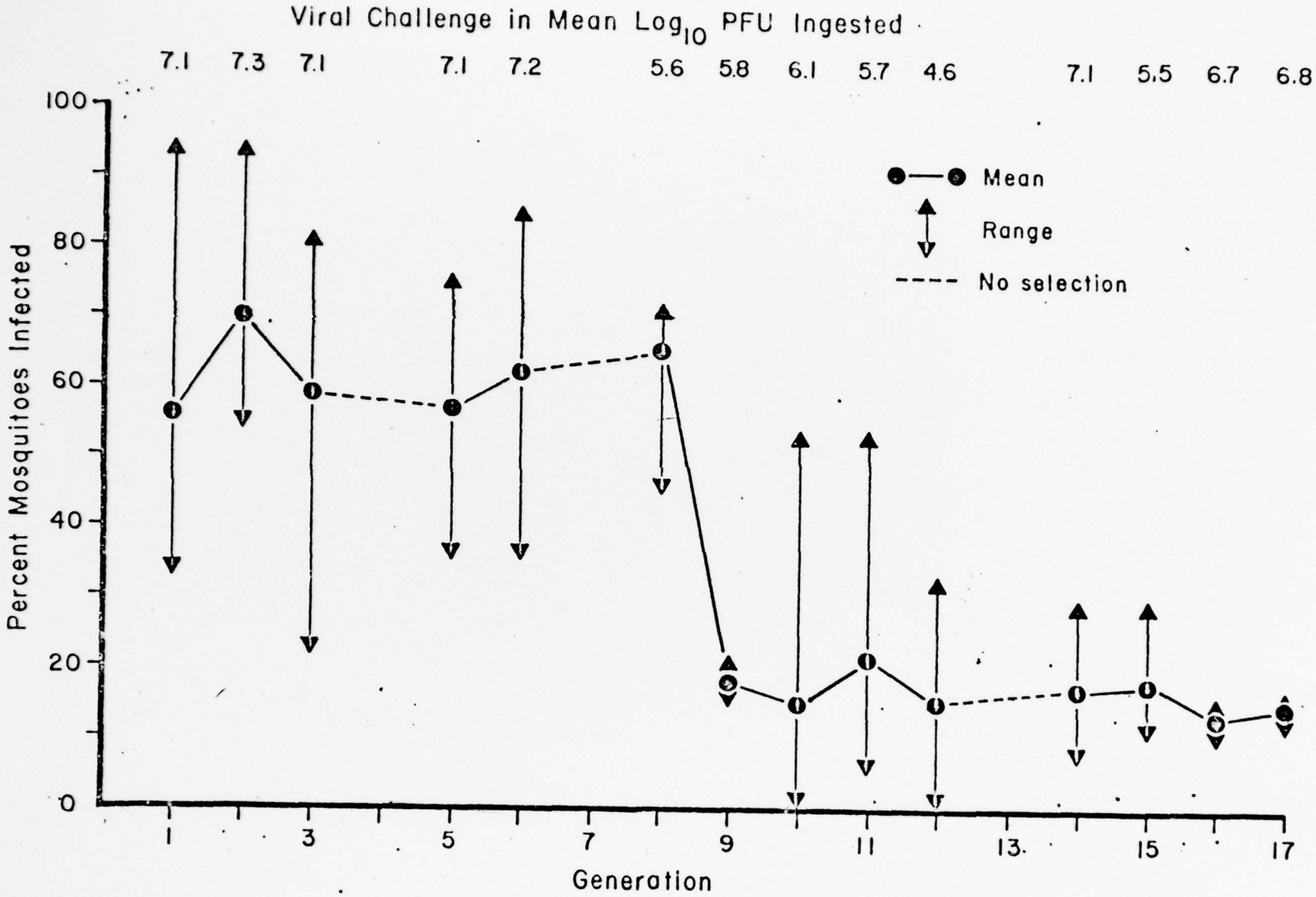
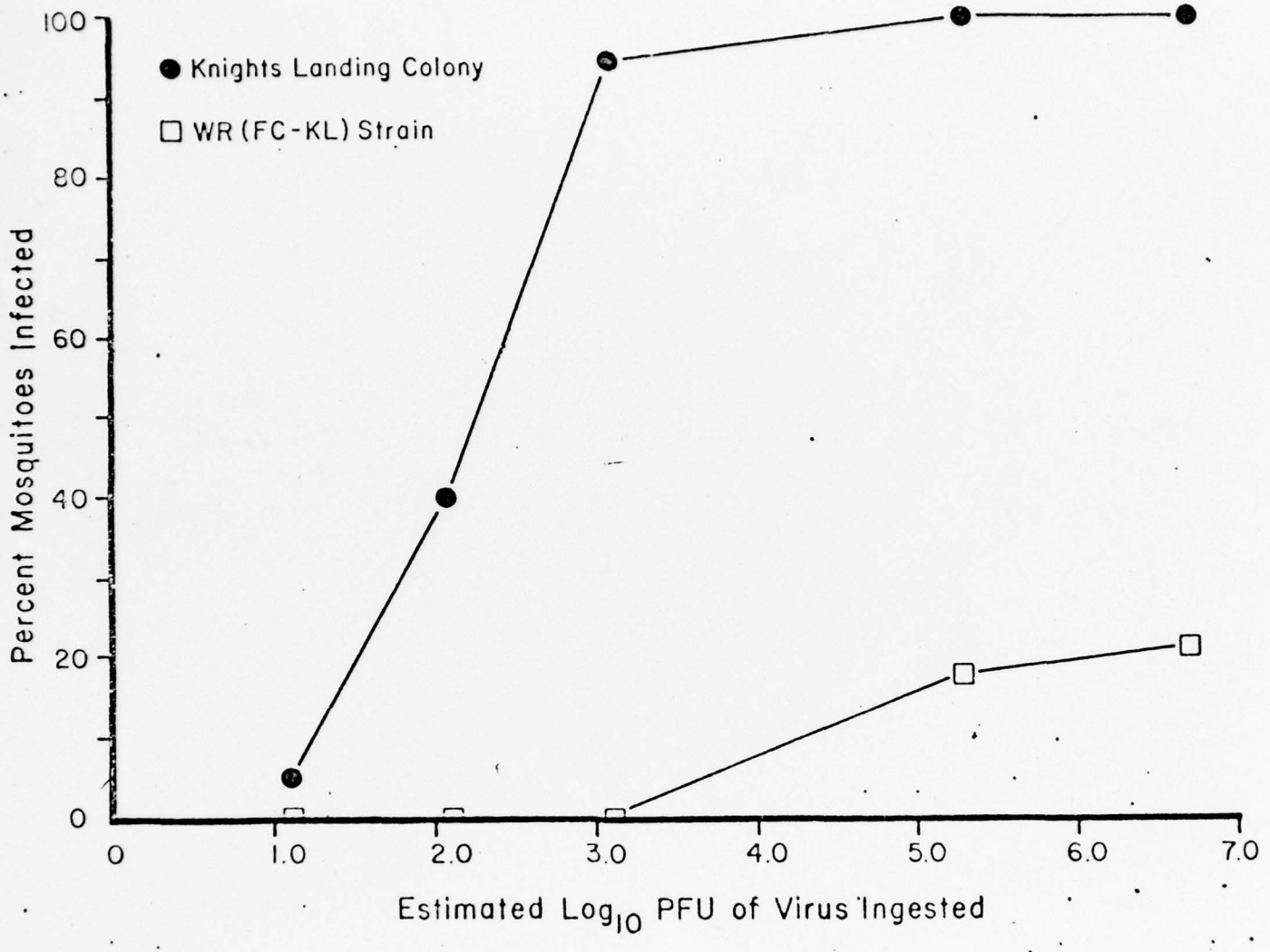


Figure 12. Profiles obtained with the highly susceptible and refractory strains when feeding on viremic chicks.



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A. Principal investigator, S. Monica Asman

Presented papers (1976-77)

"Complete marker lines for genetic studies in Culex tarsalis," 1976 Calif. Mosq. Cont. Assn. Bakersfield, California.

"Current status of the genetics of Culex tarsalis for possible application in integrated control," 1976 Inter. Cong. of Entomology, Washington, D. C.

"Competition studies of C. tarsalis stocks carrying translocations with laboratory and field populations," 1976 ESA Meeting, Hawaii.

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Asman, S.M. 1976. A preliminary study on inducing reciprocal translocations and other chromosomal anomalies in Culex tarsalis. Mosq. News 36, 58-62.

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Hardy, James L., George Apperson, S. Monica Asman, and William C. Reeves. 1977. Selection of a strain of Culex tarsalis highly resistant to oral infection with Western Equine Encephalomyelitis virus (Submitted to the Amer. J. of Trop. Med. and Hyg.).

Terwedow, H.A., Jr., S.M. Asman, P.T. McDonald, R.L. Nelson, and W.C. Reeves. 1977. Mating competitiveness of double heterozygote male Culex tarsalis in laboratory and field cage trials (Submitted to Annals of the ESA).

B. Dr. Paul McDonald (Assistant Research Entomologist I)

Presented paper (1976)

McDonald, P.T., Terwedow, H.A. and Asman, S.M. 1976. Translocations captured by genetic marker strains for genetic control of Culex tarsalis. Proc. Calif. Mosq. Cont. Assoc. 44, 62-64.

Publication in preparation

McDonald, P.T., S.M. Asman, and H.A. Terwedow, Jr. 1977. Genetics of several new translocations in Culex tarsalis (To be submitted to J. of Heredity).

Personnel receiving contract support

Dr. Paul McDonald (Assistant Research Entomologist I)(100% time)

Dr. McDonald has been on the program from its conception in 1974. Prior to that time he had three years of field experience with Aedes aegypti control in Africa.

Mr. Arvin Krueger, Research Assistant (50% time), is a pre-doctoral student in the Division of Entomology and Parasitology, Berkeley Campus.

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